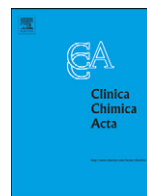




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Rapid HCP5 single-nucleotide polymorphism genotyping: A simple allele-specific PCR method for prediction of hypersensitivity reaction to Abacavir

Cristian A. Galván^{a,b}, Osvaldo C. Elbarcha^a, Eduardo J. Fernández^a,
Dante M. Beltramo^{a,c,*}, Néstor Walter Soria^{a,b,*}

^a Laboratorio de Análisis Clínicos Especializados (LACE), Córdoba, Argentina

^b Cátedra de Biotecnología, Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Córdoba, Argentina

^c Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR), Santa María de Punilla, Córdoba, Argentina

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ABSTRACT

Background: The most important factor limiting the success of an antiretroviral therapy is toxicity. The HLA-B*5701 allele is predictive of hypersensitivity reaction to Abacavir, and this gene is in a perfect linkage disequilibrium with the rs2395029 SNP present in the HCP5 gene.

Methods: Genomic DNA was extracted from blood obtained from 201 unrelated healthy Argentinean volunteers. The DNA was subjected to an allele-specific PCR method. Sequencing was performed to validate the test results.

Results: We were successful to amplify specific fragment of interest from the DNA samples. The method is easy, specific and reproducible.

Conclusions: The application of this methodology is a rapid and simple method to detect the HCP5 polymorphism (rs2395029) previous to administration of Abacavir in patients with HIV infection.

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1. Introduction

The use of highly active antiretroviral therapy in the treatment of HIV infection has resulted in significant reductions in mortality and morbidity worldwide [1]. However, there is considerable interindividual variability in patient outcomes in terms of drug disposition, drug efficacy and adverse events. The basis of these differences is multifactorial, but host genetics are believed to play a significant part. To date, most attempts to explain this variability have focused on isolate single nucleotide polymorphisms. The most exciting development to date is the discovery of human leukocyte antigen subtype B*5701 (HLA-B*5701) as a strong predictor of the Abacavir hypersensitivity reaction [2,3].

The nucleoside-analog reverse-transcriptase inhibitor Abacavir is a widely used antiretroviral drug. Approximately 5 to 9% of patients treated with Abacavir develop a hypersensitivity reaction characterized by multisystem involvement that can be fatal in rare cases [2,3]. Symptoms usually appear within the first 6 weeks of treatment and

include fever, rash, gastrointestinal symptoms, and lethargy or malaise.

The usefulness of genetic screening for the purpose of reducing the incidence of Abacavir hypersensitivity has been demonstrated in white populations [4]. Therefore, screening for HLA-B*5701 before initiation of Abacavir therapy is recommended in settings where HLA typing is available [5].

The current gold standard in screening for HLA-B*5701 is the sequence-based genotyping method. Its universal use is limited because it requires specialized laboratories and is labor intensive; in addition, its relatively high costs. Alternative HLA-typing methods include polymerase chain reaction sequence-specific primer (PCR-SSP) assay and flow cytometry [6,7]. However, none of these alternative techniques shows 100% concordance with the results of sequence-based HLA typing, and they cannot reliably differentiate between HLA-B*5701 and closely related HLA-B alleles (e.g., HLA-B*5702, HLA-B*5703, and HLA-B*5801) that are not associated with Abacavir hypersensitivity.

The rs2395029 SNP in the major histocompatibility complex P5 (HCP5) region, shows a perfect linkage disequilibrium ($r^2 = 1.0$) with the HLA-B*5701 [8]. Several studies have shown that the rs2395029 (G) allele is 99.9% predictive of the presence of an HLA-B*5701 allele [9].

In the present study, we develop a new method that is less expensive and less labor-intensive to detect the presence of the T/G polymorphism in the HCP5 gene (rs2395029) in a group of Argentinean healthy subjects from Córdoba.

* Corresponding authors at: Laboratorio de Análisis Clínicos Especializados (LACE), Avenida Vélez Sarsfield 563 (5000), Córdoba, Argentina. Tel./fax: + 54 351 4938060.
E-mail addresses: dbeltramo@yahoo.com.ar (D.M. Beltramo), nes_soria@yahoo.com.ar (N.W. Soria).

Table 1
Primer sequences used for *HCP5* genotyping (rs2395029) in the studied population.

Allele specific primers		
Forward (internal control)	5'	CCACTATTGGCCATCAAAGG 3'
Forward (specific T)	5'	ATACTGTCCAATCCCTGT 3'
Forward (specific G)	5'	ATACTGTCCAATCCCTGG 3'
Reverse (common)	5'	CCTGGTCTATCACCATCT 3'

2. Material and methods

A total of 201 apparently healthy adult blood donors, randomly selected from Córdoba (Argentina), were assayed for T/G Polymorphism (rs2395029) in the *HCP5* gene (accession No.: NT_113891.2). There were 66 men and 135 women (mean age 43.0 years, age range 18–77 years). The study was approved by the Committee of Ethics and Research from the Oulton-Romagosa and Ministerio de Salud de Córdoba and is in accordance with the Declaration of Helsinki. In all cases, the subjects were informed and their consents were obtained.

Total DNA was extracted from peripheral blood using a Wizard Genomic DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's recommendations. DNA purity was checked by taking their optical density ratio between 260 and 280 nm. DNA fragment amplifications of the *HCP5* gene were carried out by polymerase chain reaction (PCR) using our allele specific primers (see Table 1) employing a MJ Research thermal cycler. The reactions were done in two separates tubes (one for each specific allele, T or G, respectively). The reaction consisted of 80 ng of subject's DNA; 3 µl of 5× enzyme buffer; 200 µM each dGTP, dATP, dCTP and dTTP; 10 µM of each Forward and Allele specific Primer (T or G); 20 µM of Reverse Primer; 0.6 U GoTaq polymerase (Promega) and water qsp 15 µl. Cycle parameters were as follows: 94 °C for 2 min and then 40 cycles of 94 °C for 40 s, 58 °C for 40 s and 72 °C for 50 s and final extension of 72 °C for 5 min. The first forward primer is specific for the normal (T) or mutant allele (G) giving a 404-bp product; while the second one amplifies a 835-bp product from both mutant and wild-type alleles acting as an internal PCR control.

The amplified products were separated by electrophoresis in agarose gel (1.2% w/v) and visualized by UV light after ethidium bromide staining (Fig. 1).

The accuracy of the method was confirmed by sequencing 10 selected samples (5T/T and five T/G) using the forward internal control primer and the common reverse primer. G/G was not present in the analyzed population.

Allelic frequencies were determined and statistical analysis was performed using the chi-square (χ^2) test.

3. Results

The frequency of T/G Polymorphism (rs2395029) in the *HCP5* gene was evaluated in a sample of 201 healthy Argentinean subjects, representative of the population of the central area of the country. The genotypes found in this group are summarized in Table 2. The *HCP5*

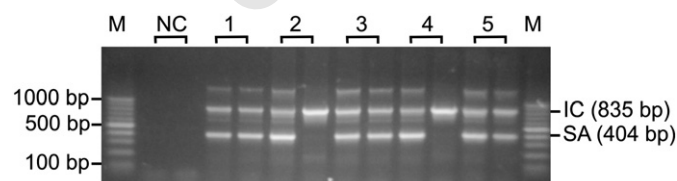


Fig. 1. Agarose gel electrophoresis. Each pair of lines represents one subject. The allele specific PCR products (left line: T allele and right line: G allele) were separated on 1.2% agarose TAE gels and sized using 1000 bp ladder. NC: PCR Negative Controls. Samples: 1, 3 and 5 had the T/G genotype; samples 2 and 4 had the T/T genotype. IC: Internal Control. SA: Specific Allele.

Table 2
Distribution of *HCP5* genotypes (rs2395029) in the studied population according to sex.

Genotype		Sex	
		n (% of total population)	
T/T	Total (%)	M	F
–/–	188 (93.5)	66 (32.8)	122 (60.7)
+/+	13 (6.5)	0 (0)	13 (6.5)
Total	201 (100)	66 (32.8)	135 (67.2)

Plus sign, mutated allele; minus sign, wild type allele; n number of subjects tested, M male, F female.

genotype distribution for T/G Polymorphism (rs2395029) was in Hardy–Weinberg equilibrium (χ^2 test: 0.22). The allelic frequencies were: T allele 0.967 (95% CI 0.898, 1) and G allele 0.033 (95% CI 0, 0.102). We have found thirteen T/G (6.5%) and 188 T/T (93.5%). T/T was the most common genotype and the G/G was not detected.

Finally, the method described here is fast and could be employed as a simple and cost-effective determination of *HCP5* polymorphism in patients with HIV infection previous to starting Abacavir treatment.

4. Discussion

In 2007, in Argentina, the National Health Authorities estimate about 134,000 people living with HIV/AIDS. We consider it necessary to develop a technique that was simple and specific that it can be used by many laboratories in order to contribute to the realization of this pharmacogenetic study, previous to the treatment with Abacavir in AIDS patients.

Many techniques currently exist to detect the presence of *HLA-B*5701* [7,10,11], which need expensive reagents and equipment. We take advantage of the perfect linkage disequilibrium between the rs2395029 SNP in the gene *HCP5* and *HLA-B*5701*, and we developed an allele specific PCR.

In this work, we determined the frequency of one genetic polymorphism in the *HCP5* gene in healthy subjects from Córdoba, Argentina. We selected a population that included 201 volunteers of both sexes, none of them younger than 18 years old. To analyze the T/G Polymorphism (rs2395029) in the *HCP5* gene, we develop a simple Allele Specific PCR amplification and separation of fragments by agarose gel electrophoresis, which allow us to obtain the results in few hours. We found that only 6.5% of people were heterozygous for T/G. The values found are in agreement with those other Caucasian populations (HapMap: http://hapmap.ncbi.nlm.nih.gov/cgi-perl/browse/hapmap27_B36/?name=SNP%3Ars2395029).

The application of this methodology is a rapid and simple method compared with others [12] to detect T/G Polymorphism (rs2395029) in the *HCP5* gene previous to administration of Abacavir.

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